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ADP019715

TITLE: Effect of Nano-to Micro-Scale Surface Topography on the Orientation of Endothelial Cells

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TITLE: Materials Research Society Symposium Proceedings. Volume 845, 2005. Nanoscale Materials Science in Biology and Medicine, Held in Boston, MA on 28 November-2 December 2004

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Effect of Nano-to Micro-Scale Surface Topography on the Orientation of Endothelial Cells

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ABSTRACT

The effect of grating textures on the alignment of cell shape and intracellular actin cytoskeleton has been investigated in bovine aortic endothelial cells (BAECs) cultured on a model cross-linked poly(dimethylsiloxane) (PDMS). Grating-textured PDMS substrates, having a variation in channel depths of 200 nm, 500 nm, 1 μ m and 5 μ m, were coated with fibronectin (Fn) to promote endothelial cell adhesion and cell orientation. As cells adhered to the Fn-coated surface, the underlying grating texture has shown to direct the alignment of cell shape, F-actin and focal contacts parallel to the channels. Cell alignment was observed to increase with increasing channel depths, reaching the maximum orientation where most cells aligned parallel to channels on 1- μ m textured surface. Immunofluorescence studies showed that F-actin stress fibers and vinculin at focal contacts also aligned parallel to the channels. Cell proliferation was found to be independent of grating textures and the alignment of cell shape was maintained at confluence.

INTRODUCTION

The application of synthetic polymers as a non-degradable vascular substitute for bypass surgery often encounters problems of thrombogenicity and long-term patency, especially for graft diameters less than 6 mm [1]. Up to date, the approach to prevent thrombosis and to improve the biocompatibility of synthetic grafts is to introduce a functional monolayer of endothelial cells onto the graft surface [1]. This requires modifications of polymer surface such as adsorption with extracellular matrix (ECM) proteins [2-4] or covalent attachment with RGD

peptide sequences [1,5-7] to promote cell adhesion. Additional to surface chemical composition, topographic textures of the surface also guides changes in the morphology and the orientation of adherent endothelial cells [4,8]. Alignment of cell shape and intracellular F-actin [8] as well as the formation of vinculin at focal adhesion sites [4] has been demonstrated in endothelial cells cultured on grating-textured surfaces. This grating-textured surface may provide an alternative method than flow in linear vessels [9] to achieve the structurally aligned and elongated endothelial cells. To investigate changes in cell morphology due to variation in channel depths, endothelial cells were cultured on grating-textured PDMS surfaces, having channel depths varied from 200 nm to 5 μm and lateral periodicity of 8 μm , and cell orientation as well as organization of F-actin and focal adhesion was evaluated.

EXPERIMENTAL PROCEDURES

Substrate Preparation

Grating patterns were first fabricated onto silicon (Si) wafers, which subsequently served as molds to replicate textures onto PDMS surfaces, using standard lift-off photolithography and reactive ion etching. Grating-textured and smooth PDMS substrates, respectively, were film casting from Sylgard 184 (Dow Corning) pre-mixed at a 10:1 ratio of base resin to curing agent on textured Si wafers and polystyrene Petri dish. PDMS was cured at room temperature for 48 h and annealed at 65°C for 3 h to ensure complete crosslinking.

For Fn adsorption, PDMS substrates were incubated in a 10 $\mu\text{g/ml}$ Fn (Sigma) solution, which was within the saturation range known to produce a monolayer coverage on a hydrophobic surface [10], at 37°C. The adsorption time was 1 h for smooth, 200-nm and 500-nm PDMS, while it was increased to 15 h for 1- μm and 5- μm textured PDMS to ensure that all channels were saturated with Fn. Followed the procedure described by Toworfe *et al.* [11], Fn coverage was estimated from spectrophotometer reading of the pre-conjugated Fn (*Fn) molecules with Oregon Green 488 fluorochrome (Molecular probe, Eugene, Oregon) on PDMS surfaces.

Cell Culture

For cell seeding, the non-specific adhesion on Fn-adsorbed PDMS substrates was blocked with 1% BSA [10] for 30 min at 37°C. BAECs were cultured in gelatin-coated flasks with Dulbecco's Modification of Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1 mg/ml glucose, 0.3 mg/ml L-glutamine, 10 µg/ml streptomycin, 10 U/ml penicillin, and 25 ng/ml amphotericin at 37°C in 95% air/CO₂. BAECs were seeded onto PDMS samples at a density of 6,000 cells/cm² in serum-free DMEM. At 4 h post seeding, the serum-free DMEM was removed and fresh DMEM containing 10% FBS was added. DMEM with 10% FBS was changed everyday for longer culture time (≥ 24 h).

Immunofluorescence

Briefly, the cells were first fixed in 4% formaldehyde for 20 min at room temperature, permeabilized with 0.1% Triton X-100 for 3 min, and blocked in 1% BSA solution for 30 min. After incubated with 1:200 mouse anti-vinculin (Chemicon, Temecula, CA) at room temperature for 45 min, the samples were washed in PBS three times, 10 min each. The samples were then incubated in 1:1000 FITC-conjugated goat anti-mouse (Chemicon) and 1:1000 TRITC-conjugated phalloidin (Sigma) for 45 min at room temperature. After the washing step, the samples were mounted onto glass microscopes and visualized by fluorescence microscope.

RESULTS

Surface Characterization

Figure 1 shows the plot of Fn surface density (ng/cm²) as a function of topographic surfaces. Across all smooth and textured PDMS, the average Fn surface density was estimated to be in the range of 300-350 ng/cm² ($p \geq 0.05$). Using fluorescence microscopy (not shown), the fluorescence intensity of pre-conjugated Fn was observed to be spatially uniform on all surfaces, including both ridges and channels, thus ensuring the homogeneous and identical Fn surface composition.

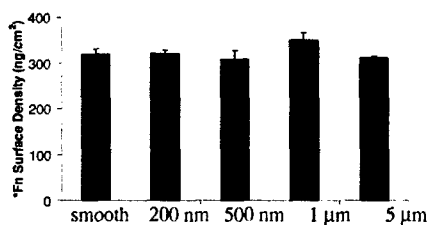


Figure 1. *Fn surface coverage on smooth and grating-textured PDMS with varied channel depths from 200 nm – 5 µm

Cell Orientation

Figure 2 shows changes in cell morphology on controlled smooth and grating-textured surfaces. Cell alignment [12], defined by the angle ($\leq 20^\circ$) formed by cell major axis and the channel direction, was observed to increase monotonically with increasing channel depths, reaching the maximum orientation on 1-µm surface. On 500-nm, 1-µm and 5-µm surfaces, the percentage of aligned cells was also strongest and significantly higher than that of non-aligned and isotropic. At confluent, cell orientation was maintained on grating-textured surfaces.

Organization of F-actin and Focal Contacts

In addition to changes in cell morphology, the intracellular F-actin and vinculin at focal contacts were also observed to align parallel to the direction of channels. Figure 3 shows the

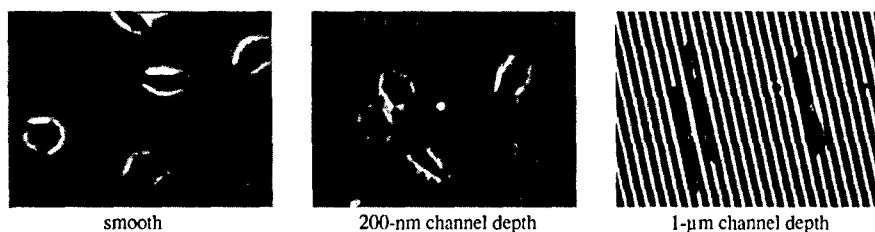


Figure 2. Phase contrast images of cell morphology on smooth PDMS and textured PDMS with 200-nm and 1-µm deep channels after 1 h seeding. Scale bar is 50 µm.



Figure 3. Fluorescence microscopy images of intracellular F-actin (red) and focal contacts (green) on smooth and grating-textured PDMS. Arrows indicates channel direction. Scale bar is 20 μm .

alignment of F-actin and focal contacts at 1 h post seeding on smooth and grating-textured surfaces. Focal contacts were shown to be preferentially located on the ridge edge, while they were randomly distributed on the smooth surface.

CONCLUSION

Endothelial cells orientation on grating-textured, chemically uniform PDMS surfaces, has shown to dependent channel depth. The effective channel depth to guide the majority of cells to align parallel to the channels was shown to be 1 μm at fixed ridge and channel widths of $\sim 4 \mu\text{m}$. Correspondingly, F-actin and focal contacts also aligned in the direction of channels.

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